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Ecology of *Micrococcus radiodurans*

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ABSTRACT

KRABBENHOFT, K. L. (Oregon State University, Corvallis), A. W. ANDERSON, AND P. R. ELLIKER. Ecology of *Micrococcus radiodurans*. Appl. Microbiol. **13**:1030-1037. 1965.—An ecological study of *Micrococcus radiodurans* indicated that microorganisms possessing the same morphological and radiation-resistance characteristics as that organism could be isolated from ground beef and from pork sausage. Further studies showed that such organisms also could be isolated from beef hides and from water from a creek adjacent to the packing plant from which the meat samples were obtained. Similar microorganisms were not isolated, however, from a limited number of samples of soil, hay, and fecal material. The use of high levels of γ-radiation in the initial isolation procedures proved to be advantageous in inactivating most of the other microflora and facilitating the isolation of *M. radiodurans*. Control experiments indicated that *M. radiodurans* did not compete well with the microflora present in ground meat, soil, and beef hides. Preincubation before irradiation of meat and soil samples or enrichment culture techniques did not enhance the isolation of *M. radiodurans*. The presence of *M. radiodurans* in creek water suggested one possible source of this organism.

The isolation of a highly radiation-resistant microorganism, *Micrococcus radiodurans*, was first reported by Anderson et al. (1956). It was found in irradiated and unirradiated ground-meat samples from an Oregon packing plant, and pure culture studies indicated that the organism was a nonsporeforming, pink-pigmented coccus occurring predominantly in tetrads with a cell diameter of 1 μ. Subsequent studies resulted in isolation of the organism from numerous meat and poultry samples procured from various sections of Oregon. It has not, however, been isolated from numerous irradiated and unirradiated fish and shell-fish samples obtained from Pacific Northwest waters and examined in our laboratories.

Murray and Robinow (1958) isolated a morphologically similar organism, which was equally radiation-resistant, as an air contaminant. A third radiation-resistant, pigmented coccus was more recently isolated from haddock tissue by Davis, Silverman, and Masurovsky (1963). These are the only vegetative organisms reported to date which are extremely resistant to radiation. Since the literature is lacking information on possible sources of these radiation-resistant organisms, a limited ecological study of *M. radiodurans* is herein reported.

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MATERIALS AND METHODS

Culture conditions. A strain of *M. radiodurans* isolated by Anderson et al. (1956) was used as a comparative organism throughout this study. Since it has been reported to be six to eight times more resistant to γ-radiation than any other known organism (Thornley, 1963), this unusual characteristic was the main criterion used in isolating it from various environments.

The standard medium used for culture maintenance was TGYM medium; this consisted of tryptone (Difco), 5.0 g; yeast extract (Difco), 1.0 g; glucose, 1.0 g; DL-methionine, 20 mg; agar (Difco), 15 g; and distilled water, 1,000 ml; final *p*H, 7.0. Stock cultures were maintained on TGYM slants incubated aerobically at 30 C for 3 days and then stored at 2 to 6 C.

The isolation medium used consistently throughout this study was designated PCNZ medium, and consisted of tryptone (Difco), NZ-Case (Sheffield Chemical Co., Norwich, N.Y.), 5.0 g; agar (Difco), 15 g; and distilled water, 1,000 ml; final *p*H, 7.0. This was the preferred medium used for the initial isolation and maintenance of a pigmented radiation-resistant micrococcus from haddock tissues as reported by Davis et al. (1963). Preliminary observations in the present study also indicated that this medium provided superior growth in comparison with PC (PCNZ without NZ-Case), Brain Heart Infusion Agar, Veal Infusion Agar, and TGYM.

Sources investigated. All investigations were conducted with samples collected from an Oregon packing plant whose meat products were the

source of original isolation of *M. radiodurans* (Anderson et al., 1956).

Ground meat. Samples of fresh ground beef and ground pork were obtained by use of sterile spatulas. The samples were packed into sterile 202 × 204 cans, and were transported from the packing plant to the laboratory packed in wet ice. Samples (20 g) of each type of meat were then packed into screw-capped, glass radiation vials (25 by 55 mm) and were placed in a holder adapted for the cobalt-60 γ-radiation source (10^5 c) at Albany, Ore. Irradiation was conducted in air at wet-ice temperatures. The total exposure dose was either 1 or 2 Mrad. Unirradiated control samples were also provided.

After irradiation, the entire contents of each glass vial were transferred to a sterile Waring Blendor and blended with 200 ml of sterile phosphate buffer (0.067 M, *p*H 7.0) for 1 min.

The unirradiated controls were examined by making serial dilutions of a 1-ml portion of the blended sample in phosphate buffer and plating them in triplicate on the isolation medium. The irradiated samples were examined by plating 5 ml of the blended sample in triplicate. All plates were incubated aerobically at 30 C for 4 to 8 days before being examined for total cell counts and for "typical" pink-pigmented colonies resembling *M. radiodurans*.

All of the pink colonies were transferred to slants, and were subsequently examined microscopically and compared with stock cultures of *M. radiodurans* for Gram-stain reaction and cellular morphology.

The main criterion used in determining the presence of organisms similar to *M. radiodurans* consisted of growing the new isolates and the stock *M. radiodurans* in TGYM broth for 44 hr at 30 C on a shaker. The cells were then harvested by centrifugation (7,000 × *g*, 20 min), washed twice, and suspended in phosphate buffer. By use of a standard curve and a Bausch & Lomb spectrophotometer, the density of the cell suspension was adjusted to approximately 10^8 cells per milliliter, and a 10-ml sample was irradiated. A radiation exposure equivalent to 700,000 rad was used for comparative purposes, since this is the reported LD_{50} dose (the exposure dose resulting in 50% survival) for *M. radiodurans* in buffer (Lee, 1963). The number of survivors was determined by plate counts made in triplicate and incubated for 72 hr at 30 C. The per cent survival was calculated and compared with that of the stock *M. radiodurans*.

TGYM broth was used as the standard culture medium, since PCNZ broth consistently produced cells which were approximately 10 times more radiation-sensitive. This latter observation was investigated more thoroughly and is reported elsewhere (Krabbenhoft et al., *in press*), as well as in latter parts of this paper.

Experiments were conducted to determine the effect of pre- and postincubation on the number of pink isolates which could be obtained from meat exposed to 2 Mrad. In a similar manner, the effect of spice additives in pork sausage and the packaging operation for both types of meat were investigated. For this purpose, the following samples were collected as previously indicated: (i) eight 20-g samples of freshly ground beef obtained directly from the meat grinder; (ii) eight 20-g samples of ground beef obtained from various parts of 10 prepacked rolls; (iii) eight 20-g samples of spiced pork sausage from prepacked rolls; (iv) eight 20-g samples of freshly ground pork sausage without spices; (v) four 20-g samples each like ii, iii, and iv above, except that the samples were incubated for 18 hr at 30 C prior to irradiation; (vi) four 20-g samples each like ii, iii, and iv above, except that the samples were incubated for 48 hr at 30 C after irradiation. All samples were irradiated at wet-ice temperature; the total exposure time was 96 min at 2 Mrad. The presence of pink micrococci in tetrads was determined as described in the previous section.

Hides of live beef animals. Since preliminary observations revealed that microorganisms comparable in cellular morphology and radiation resistance to *M. radiodurans* could be isolated from ground meat, it was decided to investigate other potential sources of this organism.

An attempt first was made to look for this microorganism on the hides and hair of live beef animals. For this purpose, sterile radiation vials containing 10 ml of PCNZ broth were prepared. Sterile cotton swabs were used in swabbing the hides of 40 different live beef animals which had been held in feeding pens for at least 5 days. Each swab was broken off individually into vials containing broth and returned to the laboratory where the vial contents were mixed for 1 min on a Vortex Jr. mixer. Once again, high levels of γ-radiation were used to facilitate the isolation of *M. radiodurans*. For this purpose, all vials were exposed to 2 Mrad at room temperature in air. The influence of pre- and postincubation on the isolation procedure was determined by placing the vials at 30 C for 0, 2, or 4 days before irradiation, or at 30 C for 4 days after irradiation. The presence of viable pigmented cells was determined by making PCNZ pour plates and incubating them at 30 C for 3 to 7 days. Microscopic examinations were made of all pink colonies which resembled *M. radiodurans*. Confirmatory identification of selected isolates was made by growing the isolates in TGYM broth, irradiating them in phosphate buffer (700 krad), and comparing their survival rate with that of *M. radiodurans*. The initial cell number was adjusted to approximately 10^8 per milliliter by optical-density measurements.

Control experiments were also conducted to determine whether irradiation of a cotton swab in PCNZ broth had a deleterious effect on cell survival. A washed 44-hr culture of *M. radiodurans* was suspended in fresh PCNZ broth. A 10-ml amount of this cell suspension was transferred to a radiation vial containing a sterile cotton swab and was exposed to 700 krad. The per cent survival was cal-

culated and compared with samples irradiated in the absence of a cotton swab.

Soil. Twenty 500-g samples of top soil were collected on three different occasions from the cattle feed-lots and grazing areas and returned to the laboratory in sterile covered cans. After the soil samples were air-dried for 36 hr, the per cent moisture was calculated to be 10% and the soil *p*H was 5.8.

After the air-dried soil had been passed through a 2-mm screen, 30 portions (5 g each) were weighed out into sterile radiation vials. To 10 of the samples were added 2.5 ml of sterile phosphate buffer (equivalent to about 5% of the soil water-holding capacity) plus 1% of the sample's weight of glucose. Ten additional samples received phosphate buffer alone, and the remaining 10 samples received no additives.

All samples received 2 Mrad, and the dose rate was 965 krad/hr. After irradiation, the samples were returned to the laboratory. Four of the ten samples containing no additives were examined immediately for the presence of pink tetracocci by making duplicate pour plates at 10^{-3} to 10^{-7} dilutions on PCNZ, and incubating the plates aerobically at 30 C for 3 to 10 days. The remaining six samples were incubated at 30 C for 6 to 10 days prior to sampling. The soil samples which had received buffer alone or buffer plus glucose were handled in a similar manner.

Enrichment culture techniques were also used. This consisted of inoculating 10-g portions of soil into DeLong culture flasks containing 100 ml of PCNZ broth and incubating on a shaker at 30 C for 44 hr. Duplicate 10-ml portions were then transferred to sterile radiation vials and exposed to 700 krad. For control tests, a 1% inoculum of a 44-hr culture of *M. radiodurans* was inoculated into the soil-broth culture and incubated and irradiated in a similar manner. A second control consisted of inoculating 8×10^3 cells per gram into dry soil and attempting to reisolate the organisms with and without exposure of the soil sample to irradiation.

Hay. Hay samples totaling 600 g were collected at random from 20 different hay bales in the cattle feeding area. The samples were collected in covered sterile containers and returned to the laboratory. Twenty samples weighing 10 g each were placed in sterile 500-ml Erlenmeyer flasks and shaken vigorously for 5 min with 200 ml of sterile phosphate buffer. The liquid was decanted into a sterile container, and subsequently 10-ml portions were transferred into sterile radiation vials. These vials were then exposed to 700 krad of γ-irradiation.

After irradiation, the samples were examined for the presence of radiation-resistant, pigmented tetracocci by making duplicate pour plates at 10^0 to 10^6 dilutions on PCNZ. The plates were examined periodically during the 3-week incubation period at 30 C.

Fecal material. Approximately 500 g of fecal material were collected at random from the floor f the cattle feeding lots. Samples of this materialo were then distributed in 5-g portions into each of 20 sterile radiation vials. These samples were exposed to 2 Mrad; exposure time was 124 min.

After irradiation, the fecal samples were examined for the presence of pigmented tetracocci as previously described.

Water. One 10-liter sample of water was collected aseptically on each of three different occasions in sterile glass carboys from each of the four sources of water used in the packing plant. These sources were as follows: (i) deep well (> 100 ft; 30 m) used for washing equipment, (ii) shallow well (60 to 100 ft; 18 to 30 m) used for washing equipment, (iii) municipal water supply used for washing equipment and meat additive, and (iv) Cox Creek which was used only for watering the livestock [the creek runs parallel to and within 25 yards (22.9 m) of the meat plant.] The samples were returned to the laboratory and examined within 24 hr for organisms similar to *M. radiodurans*.

To concentrate the microflora, the water samples were passed through Millipore filters (HA; 0.45 μ; 47 mm in diameter). However, all but the city water had to be prefiltered through a sterile filter pad (Whatman no. 1) to remove extraneous material which clogged the Millipore filter. After filtration, the Millipore filter pad was cut into four equal sections with a sterile forceps and scissors and was placed in a radiation vial containing 10 ml of sterile phosphate buffer. In a similar manner, the center portion (47 mm in diameter) of the Whatman filter paper was sectioned and prepared for irradiation. Two samples, 10 and 20 ml each, of unfiltered water from each source were also irradiated. The vial contents were thoroughly dispersed prior to irradiation (700 krad) by mixing on a Vortex Jr. mixer for 1 min.

All samples were plated out in duplicate immediately after irradiation on PCNZ at a 10^{-1} to 10^{-3} dilution; in addition, 1 and 5 ml were plated from each sample vial. During a 3-week incubation period at 30 C, all plates were examined every 4 days for the presence of pink colonies. All colonies which resembled *M. radiodurans* were picked and examined microscopically. Selected presumptive isolates were grown in broth culture, and their radiation survival rate was determined as described previously.

A control experiment was conducted to determine what effect, if any, the presence of Whatman or Millipore filter paper had on the survival rate of irradiated *M. radiodurans*.

RESULTS

Ground meat. Irradiated samples of either ground beef or pork exposed to 1 Mrad contained a total aerobic flora too numerous to count at the highest dilution used (10^{-6}); hence, subsequent experiments involved the use of higher radiation levels. The total viable plate counts obtained

TABLE 1. *Total plate count per gram of ground beef or pork sausage exposed to 2 Mrad of γ-radiation**

Isolation medium	Viable cells/g from irradiated	
	Ground beef	Ground pork
TGYM	158×10^4	15×10^4
PCNZ	178×10^4	27×10^4

* Cell counts represent the average of triplicate plates incubated at 30 C for 4 to 8 days; average of three experiments.

from meat samples exposed to 2 Mrad are shown in Table 1.

The number of pink colonies obtained from beef was five per gram; the irradiated pork samples had 20 per gram. Thus, although the total number of cells surviving 2 Mrad was 7 to 10 times greater from ground beef, a larger percentage of pink-pigmented survivors was obtained from pork sausage. These results are in general agreement with those given by Anderson et al. (1956). Unirradiated meat samples of both types contained approximately 100 to 200 pink-pigmented cells per gram.

As indicated in Table 1, PCNZ medium consistently produced the largest number of survivors. Similarly, the pigmented colonies developed in 4 days at 30 C on this medium, whereas 8 days were required for the appearance of pink colonies on TGYM medium. Previous studies by Davis et al. (1963) also indicated that PCNZ medium was the best medium for isolating a radiation-resistant micrococcus from haddock tissues. However, our observations also indicated that cells grown in PCNZ broth are 10 times more radiation-sensitive than are those grown in TGYM broth. Hence, for comparative resistance studies, all of the new isolates, as well as *M. radiodurans*, were subsequently grown in TGYM broth prior to irradiation.

Incubation of fresh ground-meat samples either before or after irradiation did not alter the number of pink isolates obtained which resembled *M. radiodurans*. Similarly, no increase in pink tetracocci was noted when spiced pork sausage or prepackaged meat was examined.

A comparison of the radiation-survival rates of several new isolates and *M. radiodurans* is given in Table 2. All of the new isolates which were radiation resistant were found to be cocci, 1 μ in diameter and occurring predominantly in tetrads with a salmon-pink pigment. These characteristics are similar to those described for *M. radiodurans*.

Pink tetracocci were also isolated from ground beef which had not previously been exposed to γ-radiation. Their radiation resistance when suspended in phosphate buffer was similar to that of *M. radiodurans* (Table 2).

TABLE 2. *Radiation sensitivities of shake cultures of Micrococcus radiodurans and several morphologically similar isolates obtained from various sources*

Source	Viable cells/ml after		Per cent survival
	0 krad	700 krad	
Beef hide[a]	208×10^6	129×10^6	62.0
Beef hide[a]	135×10^6	88×10^6	65.2
Cox Creek[b]	110×10^6	85×10^6	77.2
Cox Creek[b]	141×10^6	94×10^6	66.7
Ground beef[c]	158×10^6	82×10^6	51.8
Ground pork[c]	104×10^6	62×10^6	59.6
Ground beef[d]	167×10^6	90×10^6	54.0
Stock culture[e]	206×10^6	109×10^6	53.2

[a] Obtained by exposing a cotton swab to 2 Mrad which had previously been used to swab the hides of live beef.

[b] Obtained from creek water exposed to 700,000 rad.

[c] Obtained from meat previously exposed to 2 Mrad.

[d] Obtained from unirradiated meat.

[e] *M. radiodurans*, stock culture collection, Oregon State University.

Live beef hides. The isolation of pigmented micrococci from the hides and hair of live beef animals was readily facilitated by the use of high levels of ionizing radiation (Table 3).

The use of a 4-day preincubation period prior to irradiation resulted in an over-growth of non-pigmented aerobic sporeformers and no pink colonies. A 2-day preincubation permitted the recovery of 17 pink tetracocci. The range varied from less than 1 up to 20 cells per milliliter of the irradiated sample. When the swab samples were not incubated prior to irradiation, 114 pink isolates were obtained, and 62% of these resembled *M. radiodurans* upon microscopic examination. All of the plates made from samples which were incubated for 4 days after irradiation had a reddish-pink color, and most of the plates had more than 300 colonies.

Using isolates selected at random from non-incubated samples, their relation to *M. radiodurans* was confirmed by irradiating cell suspensions in buffer at the LD_{50} dose (700 krad). The results in Table 2 indicate at least 60% survival of these isolates under these conditions.

A control experiment indicated that the mere presence of a cotton swab in preinoculated TGYM broth during irradiation did not alter the per cent survival of *M. radiodurans*.

TABLE 3. *Total colony counts of pink-pigmented tetracocci obtained by γ-irradiation (2 Mrad) of cotton swabs used to sample hides of live beef*

Group[a]	Animal no.	Pour plate dilution					
		5:0	1:0	1:10	1:20	1:100	1:1,000
2[b]	11	NG[c]	NG				
	12	2	0	1	0	0	0
	13	1	0	0	0	0	0
	14	1	0	0	0	0	0
	15	1	1	0	0	0	0
	16	0	7	2	0	0	0
	17	2	0	0	0	0	0
	18	NG	NG	0	0	0	0
	19	NG	NG	0	0	0	0
	20	NG	NG	0	0	0	0
3[d]	21	PC[e]	PC	PC	PC	PC	PC
	22	PC	PC	PC	PC	PC	PC
	23	19	4	0	0	0	0
	24	7	1	0	0	0	0
	25	15	0	0	0	0	0
	26	6	5	0	0	0	0
	27	5	1	1	0	0	0
	28	19	9	0	0	0	0
	29	6	2	0	0	0	0
	30	10	2	1	0	0	0
4[f]	31	TNTC[g]	TNTC	TNTC	TNTC	TNTC	
	32	TNTC	TNTC	TNTC	TNTC	TNTC	
	33	TNTC	TNTC	TNTC	TNTC	TNTC	
	34	TNTC	TNTC	TNTC	TNTC	297	
	35	TNTC	TNTC	217	148	100	
	36	TNTC	TNTC	TNTC	TNTC	TNTC	
	37	TNTC	TNTC	TNTC	TNTC	TNTC	
	38	TNTC	TNTC	TNTC	TNTC	TNTC	
	39	TNTC	TNTC	12	11	1	
	40	TNTC	TNTC	TNTC	TNTC	TNTC	

[a] Samples from animals 1 to 9, comprising group 1, were incubated for 4 days prior to irradiation, followed by 7 days of incubation of pour plates at 30 C. All plates in this group were discarded because of overgrowth of nonpigmented microorganisms which were not micrococci.

[b] Samples in group 2 were incubated for 2 days prior to irradiation, followed by 7 days of incubation of pour plates at 30 C.

[c] NG = no growth.

[d] Samples in group 3 were not incubated prior to irradiation; pour plates were incubated for 3 days at 30 C.

[e] PC = pure culture of yellow-pigmented micrococci.

[f] Samples in group 4 were incubated for 4 days after irradiation, followed by 3 days of incubation of pour plates at 30 C.

[g] TNTC = colonies too numerous to count at the respective dilutions.

Soil. Repeated attempts to isolate organisms resembling *M. radiodurans* from irradiated soil samples provided negative results. Neither the use of postincubation periods of 6 to 10 days for the irradiated soil nor the presence of added nutrients (glucose) or moisture promoted the isolation of pigmented tetracocci. However, the results from a control experiment with preinoculated but unirradiated dry soil showed that, of the 8,000 cells per gram originally inoculated, 3,300 per gram could be recovered. This represented a 41% recovery rate.

After 1 week of incubation of plates made from nonincubated but irradiated soil samples, only five pink colonies were observed. Three of these developed from irradiated dry soil at a 10^{-3} dilution. Microscopic examination revealed gram-positive cocco-bacilli (0.25 by 2 μ) occurring predominantly in pairs and short chains of two or three cells each. The other colonies were obtained

from samples of soil which had been moistened to 50% of its water-holding capacity prior to irradiation. Their cellular morphology was the same as that just described.

After 3 weeks of incubation of all plates, a total of 135 pink colonies were picked and examined microscopically by use of the Gram-stain technique. Of the colonies, 97% were gram-variable rods (0.5 by 1.5 μ) existing either singly or in pairs, and 3% of the colonies were composed of yeast cells. No pigmented tetracocci were found.

The use of enrichment culture techniques likewise did not facilitate the isolation of pigmented tetracocci from soil. However, a total viable-cell count of 5×10^6 per gram was obtained after 700 krad; of these, 3×10^3 to 10×10^3 per gram were found to be gram-variable bacilli (0.75 by 1.5 μ) which occurred singly and formed pink colonies on an agar surface.

The results from a control experiment indicated that no *M. radiodurans* cells could be recovered from a preinoculated 44-hr soil-broth culture exposed to 700 krad. Attempts to isolate organisms resembling those of the inoculum from unirradiated soil-broth also were unsuccessful. The final *p*H of the soil-broth culture was 6.8.

Hay. No microorganisms which resembled *M. radiodurans* in morphology or radiation resistance could be isolated from irradiated hay samples. A total aerobic viable count of 79×10^3 per milliliter was obtained after a 10-day plate incubation at 30 C. The plates were not countable after 10 days of incubation because of bacterial overgrowth. Microscopic examination of the radiation survivors indicated that a majority were spore-formers.

Fecal material. The experimental conditions used to isolate pigmented tetracocci from irradiated fecal material gave a total aerobic viable plate count of 96×10^4 per gram. However, microscopic examination of 50 selected colonies indicated that a majority of these survivors were spore-formers, and no survivors were pigmented tetracocci. More than 6 days of incubation at 30 C resulted in plates which were overgrown and not countable.

Water samples. Incubation of pour plates made from irradiated water samples for 2 weeks indicated the presence of 10 to 25 pigmented tetracocci per milliliter. They were all obtained from creek water which had not been filtered. Attempts to concentrate these organisms by filtration were not successful, even though results of a control experiment indicated no decrease in survival rate of *M. radiodurans* irradiated in the presence of Whatman or Millipore filter paper.

When selected presumptive isolates were irradiated while suspended in phosphate buffer, they were found to possess the high degree of resistance characteristic of *M. radiodurans* (Table 2).

No microorganisms resembling *M. radiodurans* were isolated from the shallow or deep well water or from the municipal water supply used at the meat plant.

DISCUSSION

An ecological study of a microorganism similar to that isolated by Anderson et al. (1956) and designated by them as *Micrococcus radiodurans* indicated that this organism could be found in several different environments near the packing plant where the original isolation was made. It was readily isolated from ground beef and pork sausage and was found to be highly resistant to γ-radiation.

To take advantage of its great radioresistance, high levels (2 Mrad) of γ-radiation were used in the isolation procedures. This facilitated the inactivation of all of the microflora except the most resistant types. However, approximately 160×10^4 microorganisms per gram of ground beef were recovered after the irradiation treatment. About 10% of this number were isolated from irradiated ground pork sausage. These results were considered significant because, until 1958, 2 Mrad were considered to constitute an effective sterilizing dose for foods (Niven, 1958). Furthermore, earlier reports (Anderson et al., 1956) indicated that no microorganisms other than *M. radiodurans* were encountered in meat which had received 2 Mrad. In the present study, the incidence of pigmented microorganisms resembling *M. radiodurans* varied between 5 and 20 per gram of irradiated meat. Hence, these pigmented strains comprised only a small fraction of the total number of survivors. The larger total number of survivors in the present study may have been due to a larger initial number of organisms, the use of a different irradiation source, different isolation media, and a longer incubation period as compared with previous studies.

When the pigmented survivors were grown in pure culture and irradiated in phosphate buffer, their survival rate indicated a LD_{50} dose of approximately 700 krad. Microscopic examination of these pink-pigmented cultures revealed cocci (1 μ in diameter) occurring as tetrads. These characteristics were similar to those previously reported for *M. radiodurans*. On this basis, and particularly because of the unusually high radiation resistance, the new isolates were considered as *M. radiodurans*.

When selected isolates among the other nonpigmented survivors of the irradiated meat were irradiated in buffer, their resistance was less than

5% of that for *M. radiodurans*. Evidently the meat substrate served as an excellent protective medium for these organisms, and this possibly could account for the high total number of survivors. It was reported by Niven (1958) that microorganisms are much more resistant when irradiated in a food as compared with a buffered suspension.

No apparent difference in susceptibility to radiation was discernible between strains of the resistant micrococcus that had been recovered from irradiated meat and those isolated from unirradiated meat and exposed to irradiation for the first time. These observations were in accord with previous reports, and indicated that radiation resistance probably was a stable, inherent characteristic not acquired as a result of previous radiation exposures. The number of pink isolates obtained from unirradiated meat varied from 100 to 300 per gram.

Incubation of the meat samples either before or after irradiation did not increase the number of pink isolates obtained which resembled *M. radiodurans*. Evidently this organism does not compete well with other members of the microflora in this environment. The examination of spiced versus nonspiced pork sausage and fresh versus prepackaged ground beef and pork did not result in an increased number of pink isolates. This suggested that neither the spices nor the packaging operation contributed to the presence of organisms resembling *M. radiodurans* in the meat samples.

Pink-pigmented micrococci occurring in tetrads and possessing a high degree of radiation resistance in buffer were also isolated from beef hides. Once again, ionizing radiation facilitated the initial isolation of these organisms from cotton swabs which were used to brush the hides. These isolates were considered to be *M. radiodurans* on the basis of their radiation-survival rate in buffer.

Swab samples which were not incubated prior to irradiation produced 114 pink isolates, of which 62% resembled *M. radiodurans* upon microscopic examination. Only one-tenth of this number could be obtained from swabs which had been preincubated in broth for 2 or 4 days prior to irradiation. This substantiates a previous statement that *M. radiodurans* does not compete well with other microorganisms in an enriched culture medium. When the swab samples were irradiated before incubation, however, the plates containing the recovery media presented a reddish-pink color because of the abundant growth of pigmented colonies. Upon microscopic examination, a majority of these proved to be gram-positive micrococci arranged in tetrads. Hence, a postincubation period appeared to offer a good method of isolating this organism from beef hides. The results of a control experiment indicated that the survival rate of *M. radiodurans* was not altered when cell suspensions were irradiated in the presence of sterile cotton swabs. The isolation of pure cultures of yellow pigmented micrococci from two irradiated swabs suggests the presence of a new radiation-resistant microorganism. Additional studies are being conducted on these isolates.

It was not possible to isolate organisms resembling *M. radiodurans* from soil, fecal material, or hay samples. However, control experiments involving soil inoculated with *M. radiodurans* indicated that the experimental procedures allowed the reisolation of this organism from soil at a 41% recovery rate. These results suggested that microorganisms of this type were not originally present in the soil samples tested. Likewise, the use of pre- and postincubation periods and the addition of nutrients or moisture to the soil did not promote the isolation of pigmented tetracocci, although nonsporeforming bacilli which formed pink colonies were readily obtained. Evidence that *M. radiodurans* did not compete well with the microflora of a soil environment was provided from a second control experiment. By use of an enrichment culture technique, it was found that cells of this type could not be reisolated from a preinoculated 44-hr soil-broth culture. The use of radiation (700 krad) did not alter this observation.

By irradiating the water samples obtained from a creek near the packing plant, it was possible to isolate gram-positive, pigmented tetracocci which proved to be just as resistant as *M. radiodurans* when irradiated in phosphate buffer. Their incidence was 10 to 25 per milliliter of water sample after 700 krad. Attempts to concentrate these organisms by filtration techniques were unsuccessful, although control experiments indicated that the presence of Millipore or Whatman filter paper did not alter the recovery rate of irradiated cell suspensions. The creek from which the samples were obtained is used as a source of water for the livestock at the packing plant. According to the packing plant authorities, this water supply is pumped to the cattle holding pens and is used only for watering the livestock. The cattle are maintained at least 100 yards from the creek, and in no case is refuse dumped into it. The samples in which the radiation-resistant micrococci were found were obtained about 300 yards upstream from the meat plant, and they were found in five different sampling areas.

The presence of *M. radiodurans* in this environment suggested a possible source and habitat of this organism. However, additional experi-

ments should be conducted to confirm this observation and to determine whether this creek serves as an original source of this organism or whether the organisms are fed into this creek from another source.

LITERATURE CITED

ANDERSON, A. W., H. C. NORDAN, R. F. CAIN, G. PARRISH, AND D. DUGGAN. 1956. Studies on a radio-resistant micrococcus. I. The isolation, morphology, cultural characteristics and resistance to gamma radiation. Food Technol. **10**:575–577.

DAVIS, N. S., G. J. SILVERMAN, AND E. B. MASUROVSKY. 1963. Radiation-resistant pigmented coccus isolated from haddock tissue. J. Bacteriol. **86**:294–298.

LEE, J. S. 1963. Investigation of factors influencing radiation resistance of *Micrococcus radiodurans*. Ph.D. Thesis, Oregon State Univ., Corvallis.

MURRAY, R. G. E., AND C. F. ROBINOW. 1958. Cytological studies of a tetrad-forming coccus. Intern. Congr. Microbiol., 7th, Stockholm, p. 427–428.

NIVEN, C. F., JR. 1958. Microbiological aspects of radiation preservation of food. Ann. Rev. Microbiol. **12**:507–524.

THORNLEY, M. J. 1963. Radiation resistance among bacteria. J. Appl. Bacteriol. **26**:334–345.